

THAT WHICH IS CLAIMED:

1. A method of screening compounds to identify a folate antagonist having increased selectivity, said method comprising the steps of:
 - 5 (a) screening compounds to identify one or more compounds of interest that inhibit the activity of a folate-dependent enzyme; and
 - (b) screening one or more of the compounds of interest identified in step (a) to determine the level of binding of the compound of interest to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase; wherein a compound of interest that has a low level of binding to at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase is identified as a folate antagonist having increased selectivity.
- 20 2. The method of claim 1 wherein the level of binding of the compound of interest for at least one of said one or more enzymes is determined by measuring the binding affinity of the compound of interest for the enzyme.
- 25 3. The method of claim 1 wherein the level of binding of the compound of interest to at least one of said one or more enzymes is determined by measuring the activity of the enzyme in the presence of the compound of interest.
4. The method of claim 3 wherein the activity of the enzyme is measured in a cell-based assay.
- 30 5. The method of claim 3 wherein the activity of the enzyme is measured in a cell-free assay.

6. The method of claim 5, wherein the enzyme is recombinantly produced.

7. A folate antagonist identified by the method of claim 1.

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8. A method of screening for a selective folate antagonist, said method comprising:

10 (a) screening two or more folate antagonists to determine their level of binding to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase; and

15 (b) selecting the folate antagonist that has the lowest level of binding to at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase as a selective folate antagonist.

20 9. The method of claim 8 wherein the level of binding of the folate antagonist for at least one of said one or more enzymes is determined by measuring the binding affinity of the folate antagonist for the enzyme.

25 10. The method of claim 8 wherein the level of binding of the folate antagonist to at least one of said one or more enzymes is determined by measuring the activity of the enzyme in the presence of the compound of interest.

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11. The method of claim 10 wherein the activity of the enzyme is measured in a cell-based assay.

30 12. The method of claim 10 wherein the activity of the enzyme is measured in a cell-free assay.

13. A folate antagonist having increased selectivity identified by the method of claim 8.

14. A method of selecting a folate antagonist for use in therapy for a
5 neoplastic, hyperproliferative, or immune disorder, said method comprising:

(a) screening two or more folate antagonists to determine their level of binding to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase; and

10 (b) selecting the folate antagonist that has the lowest level of binding to at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase as the folate antagonist for use in therapy for neoplastic, hyperproliferative, or immune disorders.

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15. A method of identifying a folate antagonist having a reduced risk of causing adverse effects in a subject, said method comprising:

(a) screening two or more folate antagonists to determine their level of binding to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase; and

20 (b) identifying the folate antagonist that has the lowest level of binding to at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase; as a folate antagonist having a reduced risk of causing adverse effects in a subject.

25 16. A method for determining the selectivity of a folate antagonist, said method comprising determining the binding affinity of the folate antagonist for one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase.

17. A method for predicting whether a folate antagonist will have adverse effects in treatment, said method comprising determining the level of binding of the folate antagonist to one or more enzymes selected from the group consisting of 5 glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase.

18. In a method of screening for folate antagonists for use in the treatment of neoplastic, hyperproliferative, and immune disorders, an improvement comprising 10 determining the level of binding of one or more folate antagonists to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase.

15 19. In a method of screening for folate antagonists having increased selectivity, an improvement comprising determining the level of binding of one or more folate antagonists to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase.

20 20. A method of screening for drugs that may be used to improve treatment of a neoplastic, hyperproliferative, or immune disorder, said method comprising:

(a) identifying one or more compounds of interest that inhibit the 25 activity of a folate-dependent enzyme to thereby identify a folate antagonist; and

(b) screening one or more of the folate antagonists identified in step (a) to determine their level of binding to one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase;

wherein a folate antagonist that has a low level of binding to at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase is identified as a drug that may be used to improve
5 treatment of a neoplastic, hyperproliferative, or immune disorder.

21. In a method of treating a neoplastic, hyperproliferative, or immune disorder in a subject, an improvement comprising treating the subject with a folate antagonist identified by the method of claim 1.

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22. A method of determining whether a subject has an increased risk of an adverse effect as a result of treatment with a folate antagonist, said method comprising the steps of

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(a) determining a value representing the level of one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase, in a sample taken from the subject; and

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(b) comparing the value measured in step (a) with a range of values representing the level of the same enzyme in subjects who have experienced adverse effects as a result of treatment with a folate antagonist; wherein a subject having a level of at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase falling within the range of values representing the level of the same enzyme in subjects who have experienced adverse effects as a result of treatment with a folate antagonist is identified as a subject having an increased risk of adverse effects following treatment with a folate antagonist.

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23. The method of claim 22, wherein the value measured in step (a) represents the expression level one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase,

biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase in the subject.

24. The method of claim 22, wherein the value measured in step (a)
5 represents the activity level of one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase in the subject.

10 25. The method of claim 22, wherein said sample is blood.

26. The method of claim 22, wherein said sample is urine.

27. The method of claim 22, wherein the values representing the level of
15 the enzyme in subjects who have experienced adverse effects as a result of treatment with a folate antagonist are the levels of the enzyme in subjects who have developed an adverse effect as a result of treatment with methotrexate.

28. The method of claim 22, wherein the sample is taken from the subject
20 before treatment with the folate antagonist.

29. A kit for use in the method of claim 22, said kit comprising reagents for use in determining a value representing the level of one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase in a sample taken from the subject and instructions for use in a method of determining whether the subject has an increased risk of adverse effects following treatment with a folate antagonist.

30. The kit of claim 29, wherein said instructions provide a range of values representing the levels of one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin

carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase in subjects who have experienced adverse effects as a result of treatment with a folate antagonist.

5 31. The method of claim 22, wherein said adverse effect is nephrotoxicity or hepatotoxicity.

10 32. A method of determining whether a subject has an increased risk of an adverse effect as a result of treatment with a folate antagonist, said method comprising the steps of

15 (a) determining a value representing the level of one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase, in a sample taken from the subject; and

20 (b) comparing the value measured in step a) with a range of values representing the level of the same enzyme in normal subjects; wherein a subject having a level of at least one enzyme selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase, that is significantly lower than the value representing the level of the same enzyme in normal subjects is identified as a subject having an increased risk of adverse effects following treatment with a folate antagonist.

25 33. A method of selecting a therapy for a subject affected by a neoplastic, hyperproliferative, or immune disorder, said method comprising

30 (a) determining a value representing the level of one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase in a sample taken from the subject;

(b) determining whether the value measured in step (a) falls within a range of values representing the levels of the same enzyme in subjects who have experienced adverse effects following treatment with a folate antagonist; and

5 (c) selecting a therapy for the patient based on the results of step (b); wherein a therapy that decreases the risk of an adverse effect following treatment with a folate antagonist is selected when the value measured in step (a) falls within a range of values representing the levels of the same enzymes in subjects who have
10 experienced adverse effects following treatment with a folate antagonist.

34. The method of claim 33, wherein the therapy that decreases the risk of an adverse effect following treatment with a folate antagonist comprises the use of a folate antagonist having a low level of binding for one or more enzymes selected from the group consisting of glutathione synthase, pyruvate carboxylase, propionyl-CoA carboxylase, biotin carboxylase, acetyl-CoA carboxylase, and methylcrotonyl-CoA carboxylase.
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